Role of extrinsic factors in the control of clutch-size in the Blue Tit \textit{Parus caeruleus}

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Extrinsic factors that play a role in the control of clutch-size in Blue Tits \textit{Parus caeruleus} were investigated. The disruption of ovarian follicular growth, and hence the cessation of egg-laying, is caused by a stimulus generated by eggs present in the nest. The timing of this disruption varies among females during the laying period and may happen as late as after the laying of the eighth egg of the clutch; the larger the clutch, the later the disruption of follicular growth. The timing of follicular disruption is also related to ambient temperature because the proportion of female Blue Tits that cease to lay increases when higher temperatures occur toward the end of laying. It is suggested that the use of temperature to control clutch-size may be adaptive because the food peak on which parents rely to feed their chicks is likewise affected by temperature.

In birds, the control of clutch-size may involve extrinsic factors to (1) trigger the cessation of egg-laying and (2) determine the timing of this event relative to the onset of laying. The classic approach to investigate the cessation of laying has relied on egg-removal experiments to explore the role of the stimulus generated by eggs (Cole 1917, Paludan 1951). The latter issue is associated with the adaptive significance of clutch-size. Although females that lay clutches smaller than the largest observed in the population raise a smaller number of young (Boyce & Perrins 1987, Rockwell et al. 1987, Gibbs 1988), it has been shown in the Magpie \textit{Pica pica} (Högstedt 1980), Collared Flycatcher \textit{Ficedula albicollis} (Gustafsson & Sutherland 1988), Great Tit \textit{Parus major} (Petit et al. 1988) and Pied Flycatcher \textit{Ficedula hypoleuca} (Alatalo & Lundberg 1989) that, on average, females lay a clutch that corresponds to the highest number of offspring they, individually, can recruit. Therefore, in such species females control the number of eggs they lay in relation to the environmental conditions likely to prevail when the chicks are in the nest. To do so they must, at the time of laying their clutch, somehow anticipate these conditions by using environmental cues.

In Blue Tits \textit{Parus caeruleus} the way egg-laying is stopped remains poorly understood. Winkel (1970) reported that egg-removal experiments that leave either zero, one or two eggs in the nest induce females to lay significantly more eggs, but Fox (1987), who left a single egg in Blue Tit nests during egg removal, found no evidence to suggest that manipulated females laid more than the controls. Winkel (1970) also reported that egg removal after the laying of the fourth egg no longer had any influence on egg production, which led him to speculate that it was not the number of eggs in the nest, but the time since laying the first egg that was decisive. He suggested that manipulated females may only lay two or three eggs more than their normal clutch-size.

Which environmental factor(s) Blue Tits rely on to adjust their clutch-size to the prevailing conditions when chicks are in the nest is not known. Three proximate factors, food, daylength and temperature, have been investigated mostly in other temperate zone species but with few positive results so far. Additional food has been experimentally supplied to parents of 16 species (including Blue Tits) breeding in Mediterranean or colder climates (see Meijer et al. 1990 for review); in only two species did clutch-size significantly increase. Unless birds measure their natural food resources and are not fooled by the food artificially supplied, which is usually different, this suggests that food available at the time of laying is not used, at least for the majority of these species, as an indication of the food conditions likely to prevail when chicks are in the nest. Klomp (1970) pointed out that day-length cannot directly control clutch-size because, in many species showing a seasonal decline in clutch-size, clutch-size continues to decline after the summer solstice (21 June). Furthermore, experiments conducted with two groups of Common Kestrels \textit{Falco tinnunculus} demonstrated that, both under short and constant photoperiod, which in nature (April) leads to large clutches, and under long and constant photoperiod, which in nature (June) leads to small clutches, clutch-size declined markedly with the advancement of the season with virtually the same slope as in the wild (Meijer 1989). In these experiments, air temperature was also kept constant in both groups, which demonstrates that females did not use this factor to control clutch-size (Meijer 1989). Although a drop in temperature during laying is known to

In the study reported here, I performed egg-removal experiments to determine the role of eggs in causing egg-laying to stop. Fox’s (1987) experiments, which I have re-examined carefully, are reliable; I have therefore only tested whether or not females would lay more eggs if eggs were removed as laid, leaving the nest empty. I also attempted to assess Winkel’s (1970) suggestion that females cease to respond to egg removal after laying the fourth egg by starting egg removal at various stages during the laying period. I then examined the hypothesis that ambient temperature may affect clutch-size in Blue Tits because spring temperatures are known to cause significant changes in the timing of the peak of food abundance for Blue Tits (Perrins 1973).

**METHODS**

**Egg-removal experiments**

Egg-removal experiments were conducted under licence in 1990 and 1991 in Wytham Woods, Oxford. In 1990, eggs were removed daily from 35 nests of late-breeding Blue Tits (i.e. those starting to lay in the second half of the 1990 season), starting on the 1st, 2nd, 3rd or 4th day of laying and leaving the nest constantly empty. Four nests of late breeders were also manipulated toward the end of laying, after 6 (*n* = 1), 9 (*n* = 2) and 13 eggs (*n* = 1) had been laid but while laying was still going on. In 1991, four nests of early breeders (i.e. those starting to lay in the first half of the 1991 season) were manipulated after seven (*n* = 1), eight (*n* = 2) and nine eggs (*n* = 1) had been laid and while laying was still in progress. In all cases, egg removal was continued until the female stopped laying. As eggs are always laid early in the morning (around 0600h, Perrins 1979), each experimental nestbox was usually visited daily after 0900h, to avoid disturbing the laying females, but before approximately 1900h, after which the birds might be roosting. Hence, females were never in contact with an egg during the night, which is the only time during the laying period when the female is in prolonged contact with its eggs. *Hالفorn* & Reinertsen (1985) have shown that from early during the laying period, female Blue Tits uncover the eggs on coming to roost and incubate them for a short time. The rest of the night is spent off the eggs, standing in the nest cavity, until the next morning when laying occurs. Temperature probes have also revealed that, despite the evening spell in contact with the eggs, the temperature of the eggs rises only slightly when laying is taking place, suggesting only a short contact with the eggs (Hالفorn & Reinertsen 1985). Shortly after laying an egg, the female usually covers the clutch and leaves the box.

Blue Tits lay their eggs at daily intervals (but see Dhondt *et al.* 1983). None of the manipulated nests acquired an extra egg, which might have suggested that more than one female was involved. The marking patterns on all eggs collected from a nest were compared to check that only a single female was involved in laying. For all the females considered, egg patterns were sufficiently distinct to allow the use of this method. Breaks in laying sequences of experimental nests were generally twice the normal laying interval (*n* = 8), but in two cases, no egg was laid for two consecutive days (i.e. a 3-day break); both of these coincided with the start of egg removal, though the 2-day breaks did not necessarily do so. When three consecutive days were skipped (i.e. a 4-day break; *n* = 5), always as a result of the start of egg removal, subsequent eggs laid were considered to be part of a replacement clutch, because in that case rapidly growing follicles aged 1 day or more are not ovulated and almost certainly become atretic. The detection of such 4-day re-laying intervals was often hindered by delayed ovipositions of 1 or 2 days (*n* = 2 in each case) of the egg already in the oviduct at the start of egg removal. Such eggs should therefore have been laid on the morning following the day egg removal started. The occurrence of delayed ovipositions caused by egg removal was established by feeding females with Sudan dye. This dye-feeding technique (Grau 1976) was also used to determine egg formation periods in the Blue Tit (4 days, Haywood 1991). Eggs whose oviposition was delayed were often covered with a superficial chalky deposit. Similar manifestations occur in domestic hens as a result of stress, the egg being retained in the shell-gland for a period after it should have been laid (Tullett 1987).

**Calculation of daily residual temperature**

If ambient temperature affects the size of the clutch, it is likely to do so through an increase (or a decrease) in temperature (relative to the temperature she experienced earlier on) that occurs just before the female stops laying. In order to relate the daily proportion of females that stopped laying with variation in temperature (daily residual temperature), I used, for a given day, the difference between (1) the mean temperature over the last few days of the laying period and (2) the mean temperature over the preceding period of egg formation. These two periods were estimated as follow:

(1) The start of the period during which temperature might affect clutch-size was estimated by considering the timing of the change in female behaviour during laying, because this is the earliest sign indicative of the end of laying. Toward the end of the laying period, females cease covering the eggs with nest material and progressively spend more time on eggs, so reflecting the development of incubation behaviour. These changes were detected by daily recording of nest covering, presence or absence of the female in the nestbox and the egg temperature of manipulated nests. Weekly observation of nests covering or the presence of incubating females or warm eggs in the control nests revealed a similar pattern (pers. obs., Gibb 1950). This method based on manipulated
nests might overestimate the period during which temperature may influence clutch-size; however, because this would tend to reduce the difference with temperature estimates calculated over the whole period of egg formation, not increase it, computing the daily temperature residual in this way cannot exaggerate the correlation shown below. The end of the period during which temperature might affect clutch-size was assessed by considering when the critical phase of the cessation of egg-laying occurs. Assuming that temperature does influence clutch-size, it would actually affect the timing of disruption of ovarian follicular growth. Following such disruption, small yolky follicles would become atretic, but some large yolky follicles would continue to grow and would usually be ovulated. Consequently, eggs would still be laid for a few days (maximum 3 days because the youngest surviving large yolky follicle would be about 1 day old at the time of follicular disruption). Therefore, in Blue Tits, temperature cannot have an effect on the process of egg formation during the last 3 days of the laying period.

(2) The period preceding the one during which temperature might affect clutch-size was estimated by adding the period of egg formation (4 days) to the early part of the average laying period (10 days) during which no signs of incubation were detected.

The daily temperatures used in this study were the mean of daily maximum and minimum taken at the Radcliffe Meteorological Station in Oxford.

Statistical analysis

Using GLIM (Numerical Algorithms Group 1987), I carried out a logistic regression of daily residual temperature on the proportion of females that stopped laying each day in 1990. A logistic transformation was required because the number of laying females tended to increase, and then decrease, with the season. Because the event considered here is the cessation of laying, it is irrelevant to include in the analysis females that had not yet reached a stage where cessation could have occurred. The probability of this event was zero until the fifth egg was laid, since no female laid a clutch smaller than six eggs. Hence, the total number of laying females on any given day was the number of females that had already laid at least five eggs.

RESULTS

Egg-removal experiments

In 1990, six of the 39 manipulated nests were deserted either immediately after egg removal started \((n = 4)\) or after three or four eggs had been laid \((n = 2)\). These females were never seen again in the nesting boxes, unlike the others where, when laying ended, the female either started ‘incubating’ in the empty nest before the clutch was put back \((n = 22)\) or laid a new clutch in the same box \((n = 11)\). In all cases, the identity of the renesting female was established through egg colour patterns as being similar to the female that laid the first clutch. No desertion occurred in 1991.

Egg-removal experiments starting on days 1 to 4 of laying and leaving no egg in the nest were conducted only from 17 to 25 April 1990. In the 29 nests that were not deserted, the females laid on average more eggs \((11.9 \pm 2.8\) s.d.) than the 32 control females restricted to the same interval of laying date \((9.5 \pm 1.5\) s.d.). A test for homogeneity of the mean clutch-sizes of manipulated \((n = 29)\) and unmanipulated females \((n = 169)\) was carried out using the separate-slopes model (SAS Institute Inc. 1982) to correct for the effect of laying date (Fig. 1). The difference between the two groups is highly significant (Table 1).

As shown in Figure 1, some females responded to egg-removal experiments by laying larger clutches and others apparently did not. In 1990, when all manipulations were carried out in the second half of the season, the largest manipulated clutches laid were 15, 15, 19 and 17 eggs when egg removal was started, respectively, on the 1st, 2nd, 3rd and 4th day of the laying period. Egg removals (leaving no eggs in the nest) toward the end of laying did not result in an increase in clutch-size by any females in 1990; they either laid no more eggs \((n = 2)\) or just one more (two females,

Table 1. Homogeneity test of the mean clutch-sizes of Blue Tits in manipulated and unmanipulated nests controlled for the effect of laying date

<table>
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<th>Source of variation</th>
<th>d.f.</th>
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Figure 1. Seasonal declines in clutch-size of Blue Tits in manipulated and unmanipulated nests. Among manipulated nests, each egg was removed on the day it was laid until the female stopped laying, starting on days 1–4 of the laying period. □ manipulated; ■ unmanipulated nests.
Figure 2. (a) Percentage of manipulated Blue Tit nests left uncovered by the female \(n = 29\); (b) Percentage of manipulated nests where either the female was sitting on the nest or the unoccupied nest contained a warm egg \(n = 29\).

Each of which had a clutch-size of 10 eggs). However, all four females started laying new clutches. The earliest first egg of a second clutch was laid 5 days after the removal of the first clutch. In 1991, four nests of early breeders were manipulated after seven, eight, eight and nine eggs had been laid. The final clutch-sizes of these females were, respectively, 11, 30 (in 32 days), 12 and 10 eggs.

To sum up, some females responded to egg removal starting on Days 1, 2, 3, 4 and 8 of laying by laying a larger clutch than controls, whereas others did not (8 out of 11 manipulated females, 3/4, 4/7, 4/7 and 1/2, respectively).

**Incubation behaviour during laying**

The timing of the change in the female’s behaviour during laying must be evaluated to calculate daily residual temperature. Toward the end of laying, the proportion of uncovered nests rose from 3.4% 6 days prior to the day on which the last egg was laid to 86.7% on the last day (Fig. 2a). A similar trend was observed in the attendance of the female at the nest or, if she was absent, the presence of a warm, uncovered egg; the proportion of incubated nests rose from 3.5% on the 5th day prior to the day on which the last egg was laid to 56.7% on the last day of laying (Fig. 2b). Therefore, in terms of nest covering behaviour and willingness to spend more time sitting on the freshly laid egg, the female’s behaviour began to change around the 6th day before the day the last egg was laid. Virtually no incubation occurred before that stage. Thus, the period during which temperature can presumably influence the timing of disruption of ovarian follicular growth ranged from the 6th day to the 3rd day before the last of the laying period. Daily residual temperature used in this study was therefore the difference between the mean temperature over the 6-3 days preceding the last egg date and the mean temperature over the 13-7 days preceding the last egg date (Fig. 3).

**Effect of temperature**

The seasonal variations in temperature and in the proportion of females that stopped laying are shown in Figure 4. Females laid eggs (ranked 6 or more) over 27 days. Each day, an average of 24.4 ± 13.4 s.d. females were laying, and an average of 5.2 ± 2.8 s.d. females stopped laying. On two occasions, no females stopped laying on a given day (Fig. 4). Twice, the proportion of females that stopped laying reached unity (Fig. 4): this resulted from the small number of females involved in laying \(n = 1–2\) toward the end of the season. Figure 5 shows that the proportion of females that stopped laying increased significantly with increase in daily residual temperature \(y = -1.451 + 0.095x, \chi^2 = 6.013, P < 0.05\).

**DISCUSSION**

The results of these egg-removal experiments show that most female Blue Tits require a prolonged contact with their clutch before they stop laying and to achieve a normal clutch-size; this species is an indeterminate layer (Cole 1917, Haywood 1993). Fox’s (1987) finding showed that a single egg left in the nest had the same effect as a whole clutch in being sufficient to induce the female to lay in a determinate manner because none of his manipulated females laid more than an average clutch (control clutch-sizes: 9.59 eggs \(n = 27\);
controlled clutch-sizes: 9.80 eggs \( [n = 10] \), \( t_{15} = 0.395, P > 0.05 \). Therefore, only an empty nest induces a female Blue Tit to lay more eggs.

Late-laying females, which lay smaller clutches, showed the lowest tendency to produce extra eggs. A similar result has been found in the House Wren *Troglosytes aedon* (Kendeigh et al. 1956, Kennedy & Power 1990), the American Kestrel *Falco sparverius* (Porter 1975) and the Sparrowhawk *Accipiter nisus* (Newton 1986).

Winkel (1970) found that egg removal had no effect after the fourth egg was laid and suggested that manipulated females can only lay two or three eggs more than their normal clutch-size. Despite a relatively small sample size, my observations show that females can delay the disruption of ovarian follicular growth until after laying the eighth egg and respond to egg removal by laying much more than their normal clutch-size (about twice as much). Females laying smaller clutches later in the season trigger the disruption of follicular growth earlier; some late breeders might not even need the stimulus generated by eggs to lay what appears to be the 'normal' clutch-size. Thus, in contrast with the Zebra Finch *Taeniopygia guttata*, which lays four to six eggs per clutch but shows no individual variation in the timing of follicular disruption in relation to clutch size (Haywood 1991), the timing of disruption in Blue Tits varies among females: the smaller the clutch, the earlier in the laying period the disruption of follicular growth is triggered.

The timing of cessation of egg-laying is also influenced by ambient temperature; the proportion of female Blue Tits that ceased to lay daily increased when higher temperatures occurred toward the end of laying. Kendeigh (1941) has suggested a similar effect of temperature on clutch-size in the House Wren, but his data were not corrected for the seasonal increase in temperature, so they may only reflect a non-causal correlation between the seasonal increase in temperature and the decline in clutch-size that occurred with the advancement of the season.

Spring temperature may cause significant changes in the timing of the peak of food abundance for Blue Tits (Perrins 1973). Tits adjust their timing of breeding to the peak abundance of caterpillars on which they rely to feed their young, but a sudden rise in temperature during the breeding season after the birds have started laying may cause an advance in the peak of caterpillars of up to 2 weeks (Perrins 1970, Perrins & Mc Cleery 1989). In accordance with the present findings, a rise in temperature would induce tits to stop laying earlier than expected and to start incubating the clutch as soon as possible, resulting in two advantages to the parents: (1) a reduction in clutch-size and hence brood-size and (2) the brood hatching earlier than it would have if a large clutch had been hatched.


REFERENCES


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